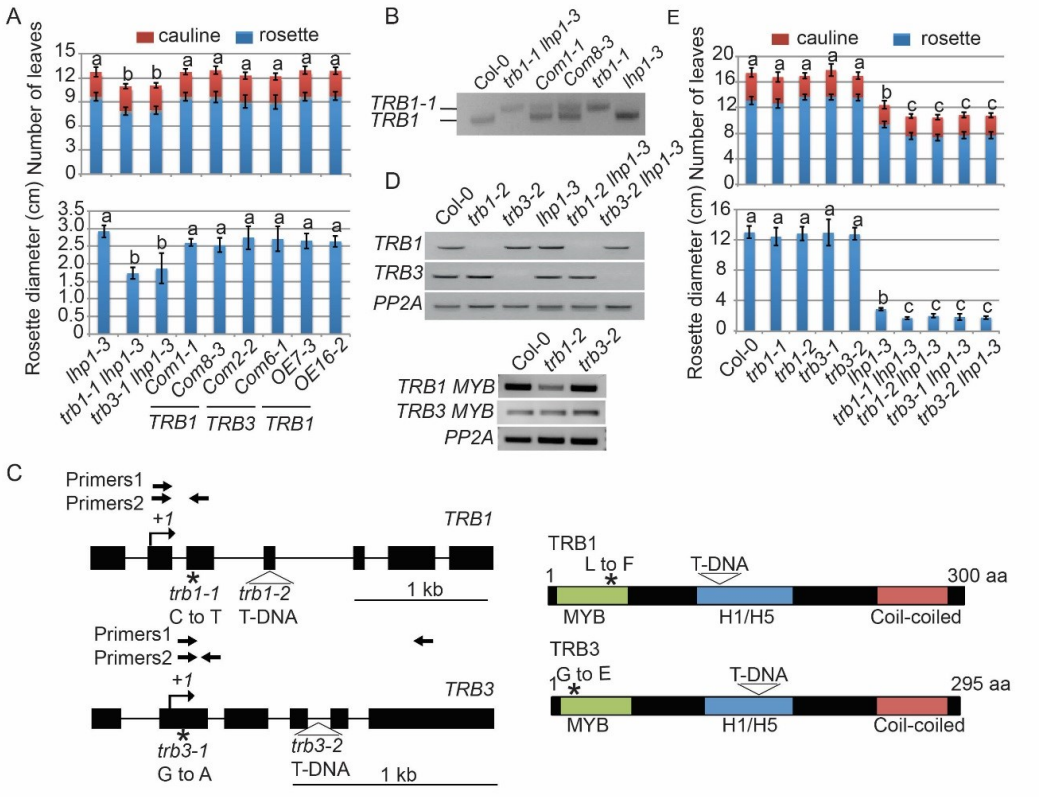
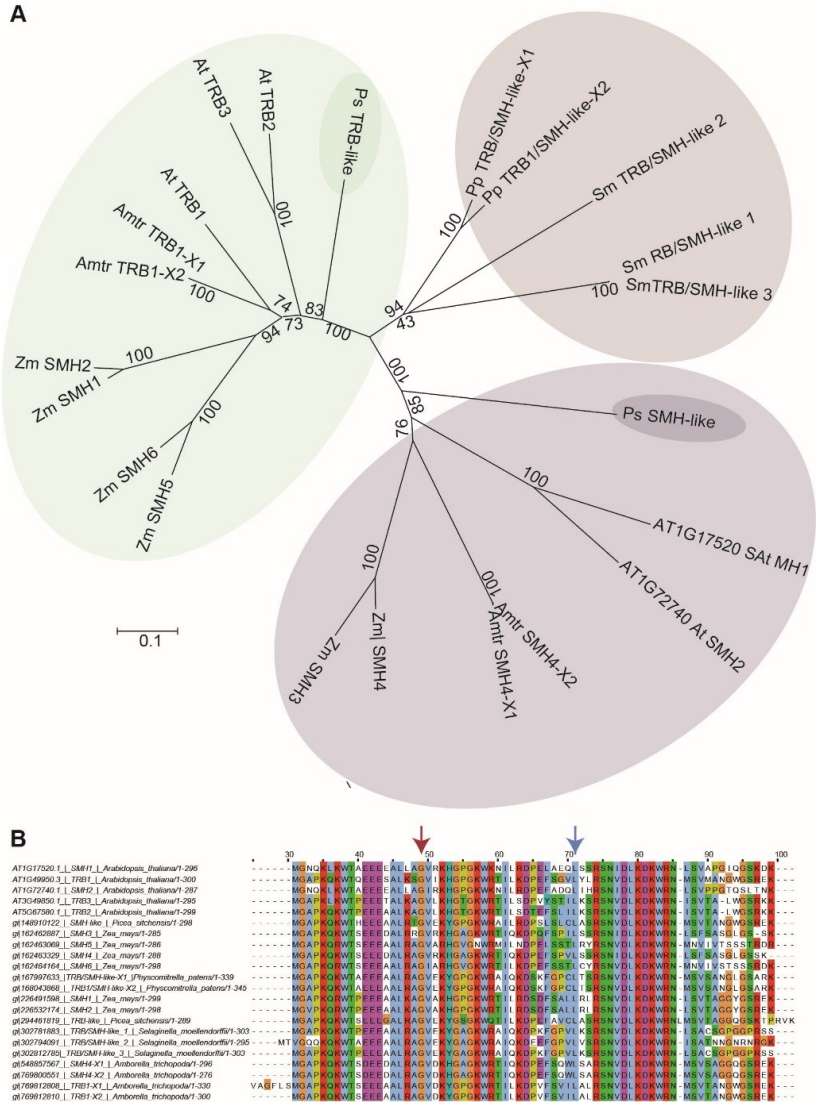



**Supplemental Figure 1. Confirmation of mutant mapping results. (A)** Complementation assay with stably transformed genomic fragments (Com*N-N*) (2 kb upstream of TSS and 1.5 kb downstream of TES) and CaMV *Pro35S:TRB1:GFP* (OE*N-N*) in corresponding double mutant backgrounds. Two independent lines per transgene are shown. Flowering time (top) and rosette diameter are shown as average (n=9). Error bars indicate s.d. (n=9). Statistical significance was determined by one-way ANOVA with multiple comparison correction by TukeyHSB. Different letters indicate significance groups ($p<0.001$). **(B)** Expression of the transgene was confirmed using a differentially Cleaved Amplified Polymorphic sequence (dCAPs) assay after amplification of cDNA prepared from total RNA. **(C)** Schematic representation of the *TRB1* and *TRB3* loci. On the left side, exons are indicated by black boxes, introns by lines, the ATG start codons (+1) are indicated by right-angled arrows. Positions of induced point mutations are indicated by asterisks, and the position of T-DNA insertions by triangles. Primer positions for transcript detected are indicated by arrows. On the right side, protein domain structure, the position of the point mutations and the T-DNA insertions codon are indicated. **(D)** Expression of *TRB1* and *TRB3* in different genetic backgrounds measured by RT-PCR. *PP2A* was used as reference gene. Presence of full-length (top) and partial (bottom) transcripts was analyzed. **(E)** Phenotype of EMS-induced *trb1-1* and *trb3-1* and T-DNA *trb1-2* and *trb3-2* lines in the Col wild-type and *lhp1-3* mutant background analyzed as in (A). Scale bars: 1 cm.



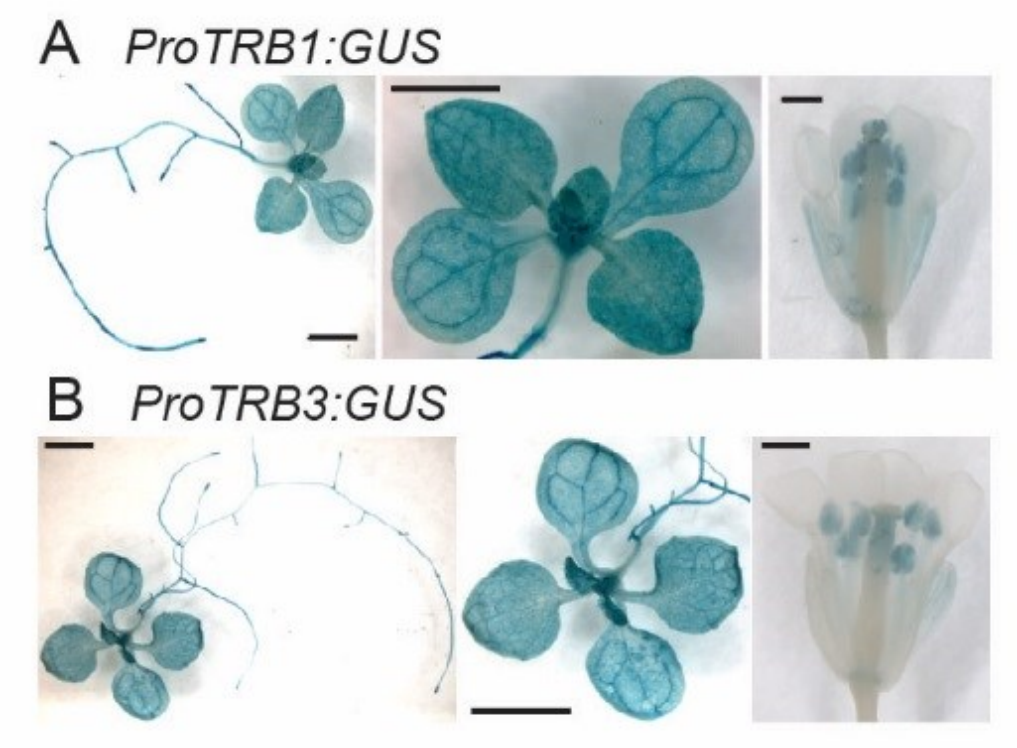
1




**Supplemental Figure 2. Phylogenetic analysis of TRB domain relatives in plants.** TRB1 was used to blast protein databases for the presence of domain relatives in selected plant species representing the land plant lineages. Only close homologs with a single repeat amino-terminal Myb and a carboxy-terminal H1/5 related domain were considered. The sequences were aligned using the MUSCLE algorithm. Phlyogenie was calculated as Neighbor-Joining tree. Quality of the tree was validated by calculating 1000 trees of bootstrapped sets using MEGA5. Bootstrap values are indicated at each branch point. Scale bar indicates genetic distance (substitutions per site). Alignments used to generate this phylogeny are presented in Supplemental Data Set 2. (B) Alignment of the single repeat Myb domains of analyzed proteins. Position of amino-acid changes in the trb1-1 and trb3-1 alleles is indicated by blue and red arrows, respectively. Numbers indicate position of amino-acids of the consensus sequence.



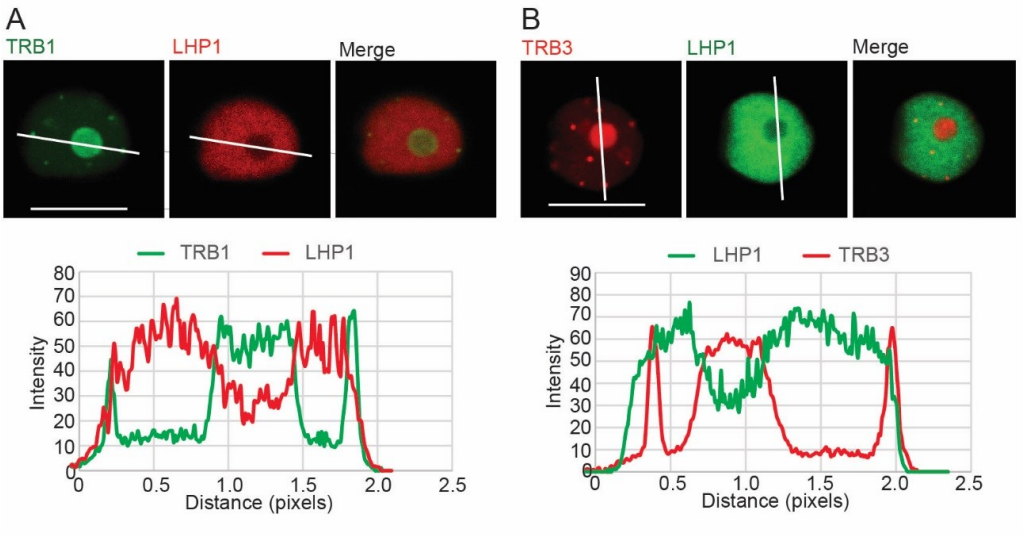
2




**Supplemental Figure 3 Histochemical detection of GUS activity for TRB1 or TRB3. (A and B)** Histochemical detection of GUS activity in 10-day-old seedlings stably transformed with *ProTRB1:GUS* (A) and *ProTRB3:GUS* (B) in Col-0 background. Scale bars are 1 mm (left and middle panels) and 0.5 mm (right panels).



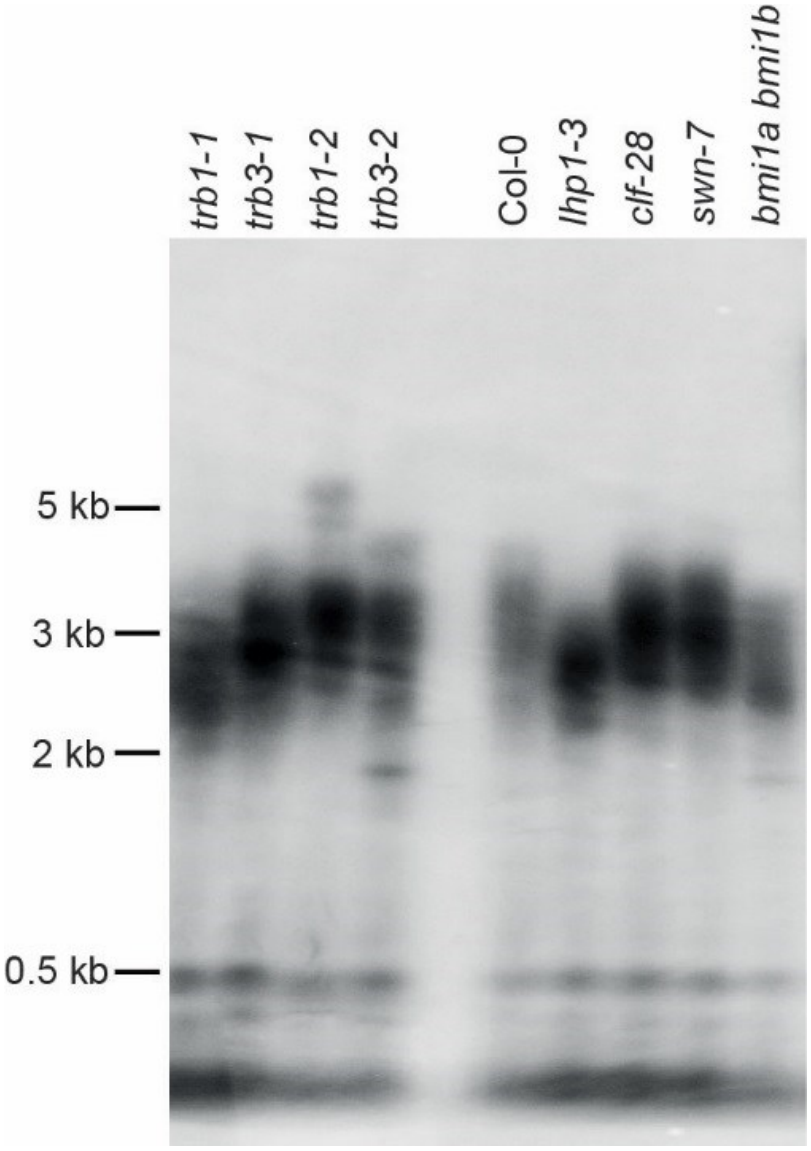
3




**Supplemental Figure 4 Colocalization of TRB1 or TRB3 with LHP1.** Top panels show confocal slices for TRB1:GFP and LHP:RFP (left) and TRB3:RFP and LHP1:GFP (right). Lower panels show analysis of pixel density along the axis indicated by a white line in the top panel performed by ImageJ. GFP signal intensities are shown in green and RFP signals in red. While scale bars indicate 10 μM.



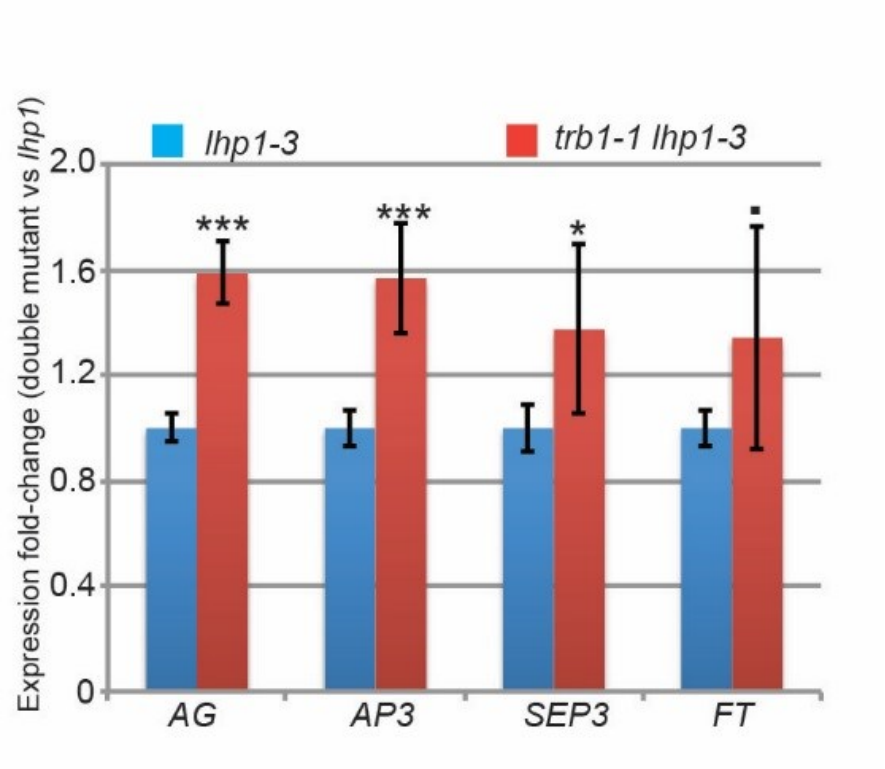
4




**Supplemental Figure 5. Telomere length in PcG-pathway mutants.** Telomere length measured by TRF analysis of genomic DNA prepared from 10-day-old Col-0, *lhp1-3*, *trb1-1, trb3-1, trb1-2, trb3-2, clf-28, swn-7* and *bmi1a bmi1b* seedlings.



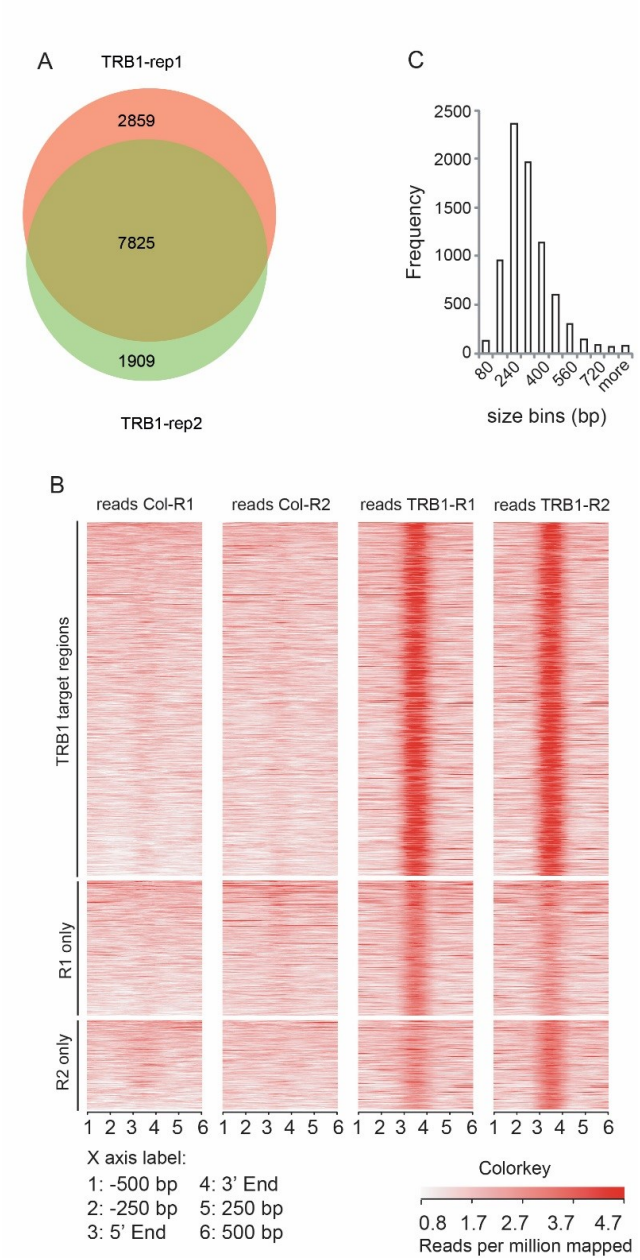
5




**Supplemental Figure 6. Quantitative RT-PCR analysis of expression levels of TRB1 target genes in *lhp1-3* and *trb1-1 lhp1-3* background.** RNA was extracted from 10 day-old seedlings grown at 22°C in LDs. Value was normalized to the reference gene PP2A and is displayed as fold difference to the *lhp1* mutant. Bars display the mean of three biological replicates, and error bars indicate ± s.d. Statistical significance was determined by Student's *t*-test (***p < 0.01, **p < 0.01 and *p < 0.05, ▪p < 0.1).



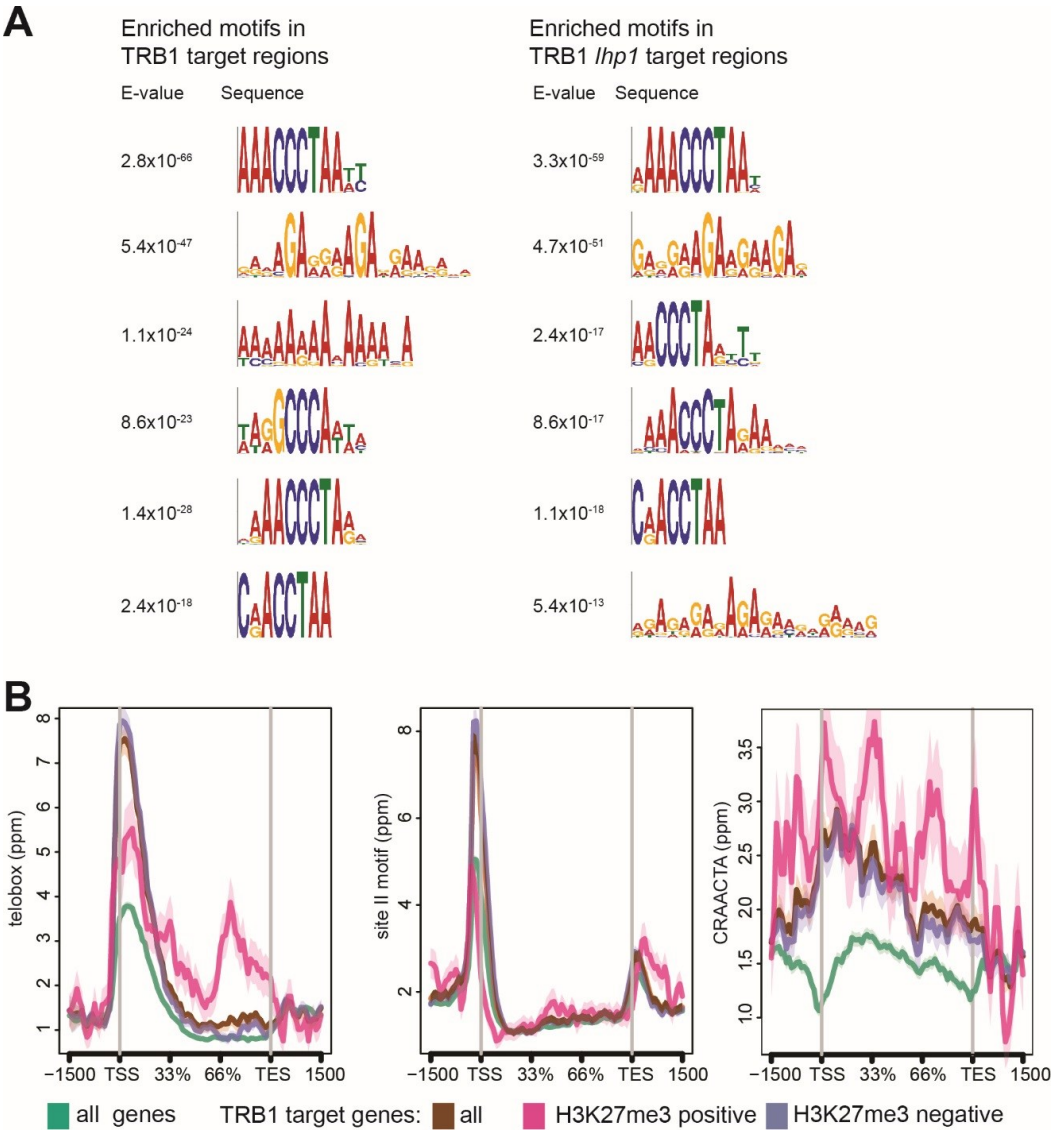
6




**Supplemental Figure 7. ChIP-seq analysis of TRB1 target regions. (A)** Overlap between target regions identified in two biological replicates. **(B)** Read coverage across centered TRB1 target regions and 500 bp flanking regions for two biological replicates of each Col control and TRB1:GFP ChIP-seq. **(C)** Histogram of length distribution of TRB1 target regions.



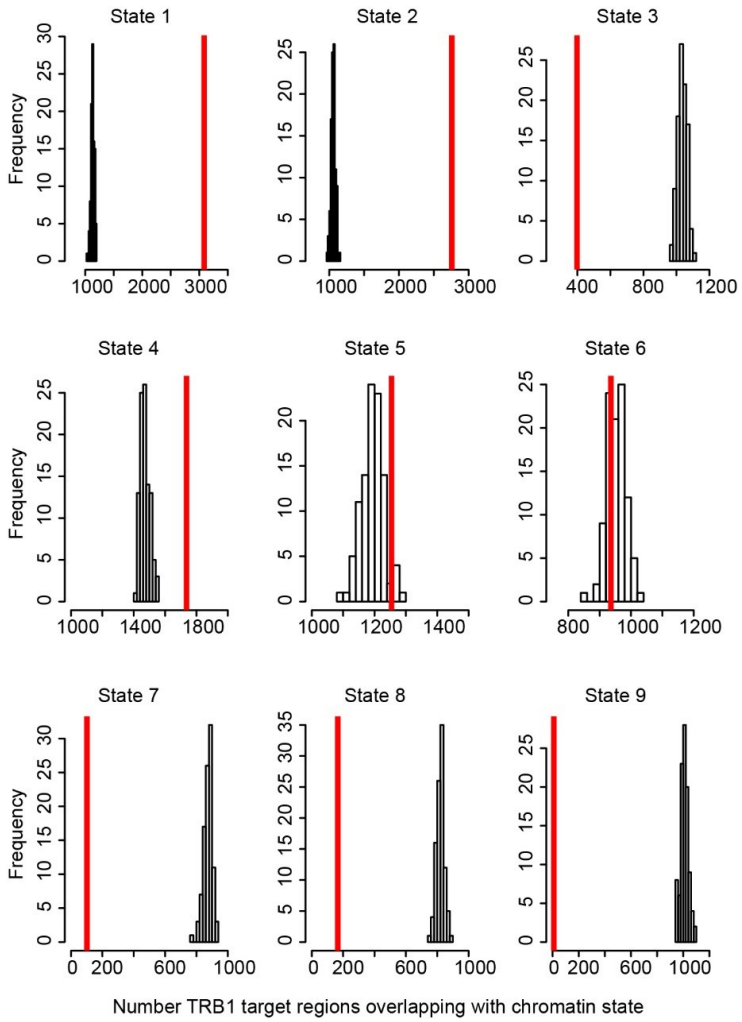
7




**Supplemental Figure 8. Analysis of *cis*-elements enriched under TRB1 ChIP-seq enriched regions. (A)** Sequence logos of the six most enriched elements of TRB1 (left side) and TRB1 *lhp1* (right side) enriched peaks detected by MEME-ChIP analysis. **(B)** Metagene analyses of *cis*-element distribution over TRB1 target genes either positive (pink) or negative (violet) for H3K27me3. Analyses of all genes (green) and all TRB1 target genes (brown) is shown as reference.



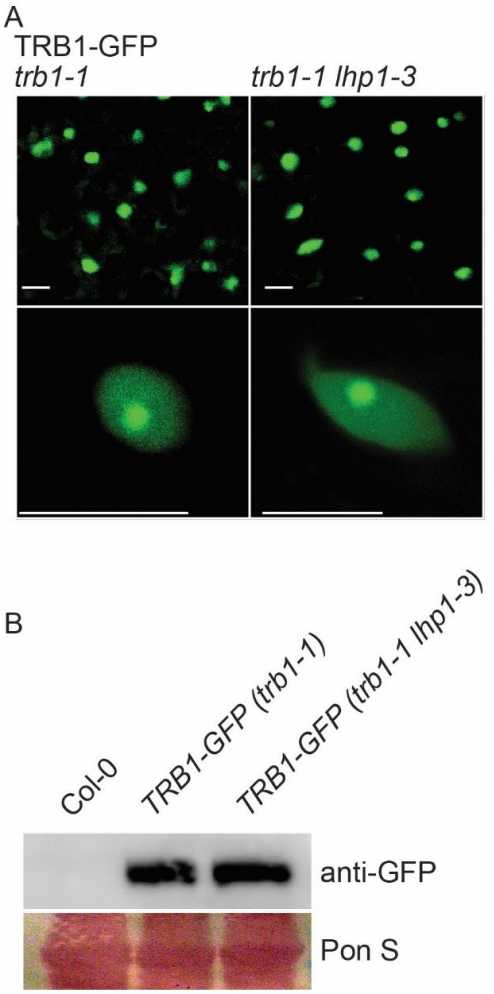
8




**Supplemental Figure 9. Overlap of TRB1 enriched regions with chromatin topology.** Nine distinct chromatin topographies were previously mapped and annotated in Arabidopsis seedlings (Sequeira-Mendes et al., 2014). Overlap between TRB1 enriched peaks and chromatin states was calculated (red line) and compared to a distribution obtained with 100 sets with randomly reshuffled binding peaks (histogram).



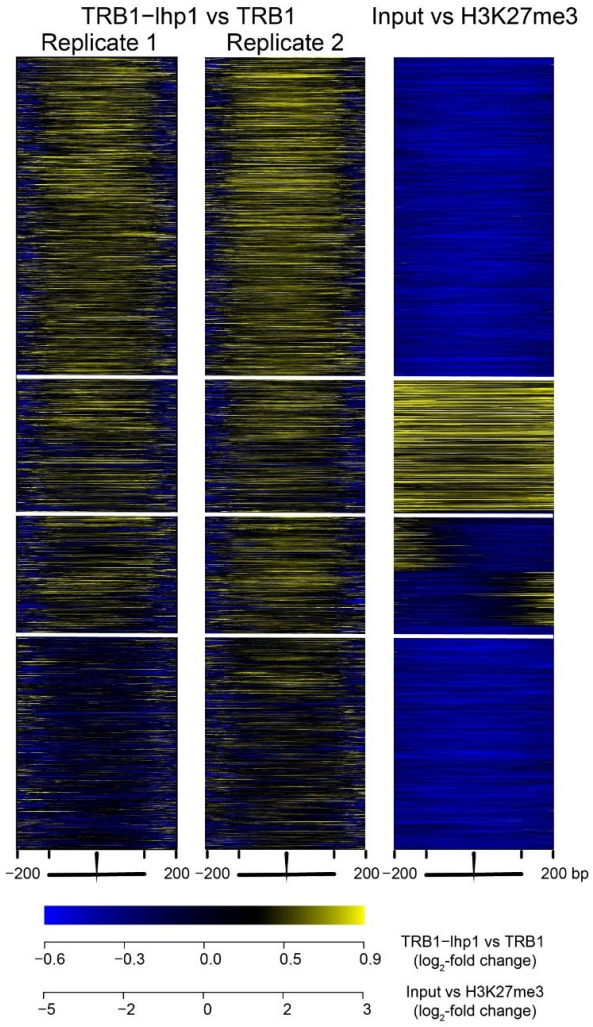
9




**Supplemental Figure 10. TRB1-GFP localization and level in *trb1-1* and *lhp1*. (A and B)** Localization (A) and protein level (B) of *TRB1-GFP* in *trb1-1* and *trb1-1 lhp1-3* backgrounds. Scale bars are 10 μm; Ponceau staining (Pon S) shows equal loading.



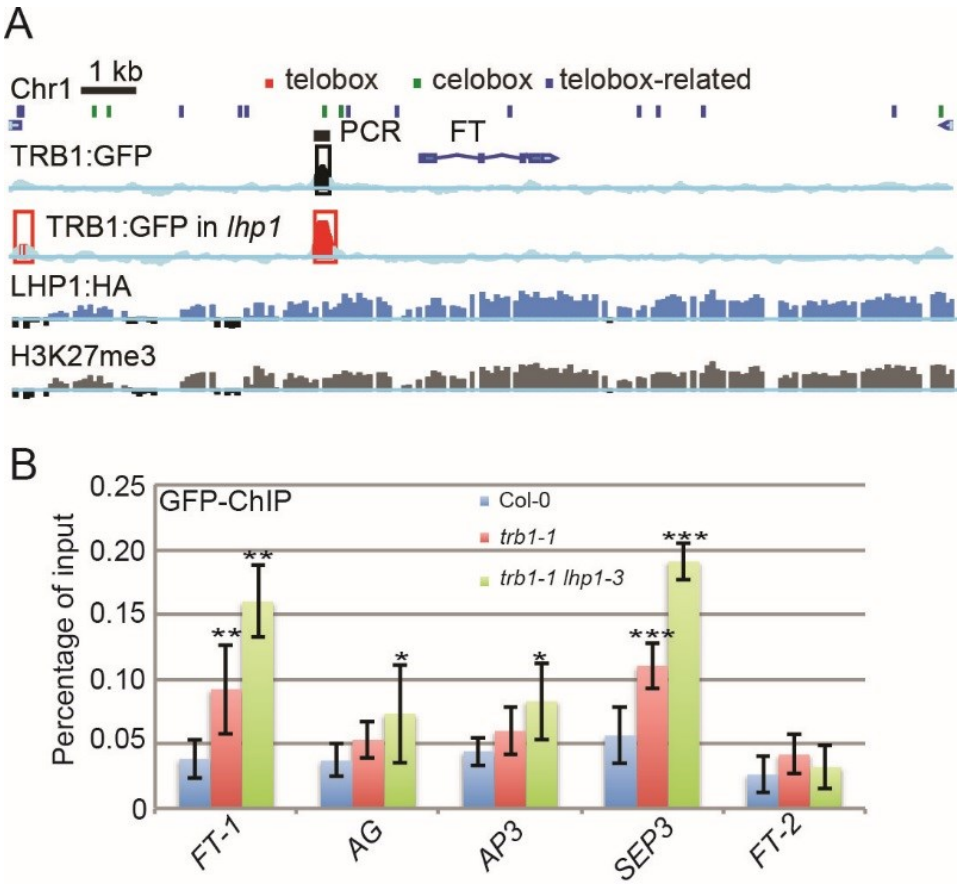
10




**Supplemental Figure 11. ChIP-seq analysis of TRB1 target regions.** Heatmap representation of differences in read coverage between genotypes. Differences were calculated for each biological replicate as $log_2$-fold change between samples after normalizing the overall number of mapped reads for each sample (left and middle panel). H3K27me3 coverage was determined by ChIP-seq and coverage calculated as $log_2$-change compared to input. Genomic fragments corresponding to the peak region and 200 bp flanking region on both sides were clustered in 4 k-means clusters based on their pattern. Clusters are separated by a white line. Due to the random orientation of fragments, H3K27me3 reads flanking the binding sites may appear on the left or right side of the peaks. Peak regions are centred to their middle (indicated by transversal line on the scale) and plotted relative to the total length of the enriched peak. Flanking regions are depicted on a bp scale. Note that the heatmap color scales are different for TRB1 and H3K27me3 ChIP-seq samples.



11




**Supplemental Figure 12 ChIP-seq and ChIP PCR binding of TRB1. (A)** Overview of the *FT* locus. Top panels show gene models with exons and introns illustrated by boxes and lines, respectively. UTRs are depicted by lighter blue fill color; direction of the coding strand is indicated by the arrow. Location of *telobox*-related-, *telobox*- and *celobox*-motifs are indicated by blue, red and green boxes, respectively. Middle panels show coverage of TRB1:GFP and TRB1:GFP *lhp1* corrected by coverage from Col control precipitation. Values more than 50 reads over background are indicated in black for TRB1:GFP and red for TRB1:GFP *lhp1*. Black and red boxes indicate location of fragments indicates significantly enriched by SICER (FDR<0.0001). Two bottom panels show ChIP-chip enrichment of LHP1:HA and H3K27me3 from our previously published data (Dong et al., 2012; Engelhorn et al., 2012). **(A)** TRB1 occupancy at *FT, AG, AP3* and *SEP3* as determined by ChIP-PCR using anti-GFP antibodies on chromatin prepared from Col-0, *TRB1:GFP* (*trb1-1*) and *TRB1:GFP* (*trb1-1 lhp1-3*) seedlings. Error bars indicate mean ± s.d. calculated from three biological replicates. Significant difference compared to WT was determined by Student's *t*-test (***p < 0.001, **p < 0.01, *p < 0.05).



12

| Mapping of *trb3-1* | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Chr** | **Position** | **Ref. base** | **SNP** | **No reads** | **Concordance** | **Score** | **Annotation** | **Gene** | **Protein variant** | **Protein position** | **Type** | **Ref. aa** | **Change aa** |
| 3 | 11523094 | G | A | 38 | 0.81 | 36 | intergenic | | | | | | |
| 3 | 12425773 | C | T | 39 | 0.83 | 36 | intergenic | | | | | | |
| 3 | 12853490 | G | A | 46 | 0.74 | 25 | intergenic | | | | | | |
| 3 | 14938576 | C | T | 42 | 0.93 | 40 | intergenic | | | | | | |
| **3** | **14951003** | **G** | **A** | **26** | **0.84** | **36** | **CDS** | **AT3G42870** | **AT3G42870.1** | **237** | **Non syn** | **W** | ***** |
| 3 | 15817542 | G | A | 37 | 0.82 | 36 | intergenic | | | | | | |
| **3** | **16159054** | **G** | **A** | **53** | **0.91** | **40** | **five_prime_UTR** | **AT3G44570** | | | | | |
| 3 | 16811122 | G | A | 43 | 0.91 | 38 | intergenic | | | | | | |
| 3 | 16850231 | G | A | 55 | 0.95 | 40 | intergenic | | | | | | |
| **3** | **18489506** | **G** | **A** | **36** | **0.88** | **36** | **CDS** | **AT3G49850** | **AT3G49850.1** | **19** | **Non syn** | **G** | **E** |
| **3** | **20828705** | **G** | **A** | **58** | **0.85** | **36** | **CDS** | **AT3G56130** | **AT3G56130.1** | **240** | **Non syn** | **E** | **K** |
| **3** | **20885609** | **G** | **A** | **44** | **0.85** | **36** | **CDS** | **AT3G56310** | **AT3G56310.1** | **393** | **Non syn** | **S** | **N** |
| **3** | **23373027** | **G** | **A** | **35** | **0.71** | **25** | **three_prime_UTR** | **AT3G63260** | | | | | |
| **Mapping of *trb1-1*** | | | | | | | | | | | | | |
| 1 | 11553564 | G | A | 19 | 0.7 | 25 | CDS | AT1G32120 | AT1G32120.1 | 213 | Syn | E | E |
| 1 | 16104383 | G | A | 19 | 0.73 | 30 | intergenic | | | | | | |
| 1 | 16546096 | G | A | 31 | 0.76 | 25 | intergenic | | | | | | |
| **1** | **16849629** | **G** | **A** | **26** | **0.76** | **25** | **CDS** | **AT1G44446** | **AT1G44446.1** | **298** | **Non syn** | **P** | **L** |
| 1 | 17099388 | G | A | 16 | 0.76 | 25 | CDS | AT1G45180 | AT1G45180.1 | 18 | Syn | E | E |
| 1 | 17391386 | G | A | 35 | 0.97 | 40 | intergenic | | | | | | |
| **1** | **17424909** | **G** | **A** | **32** | **0.94** | **40** | **CDS** | **AT1G47490** | **AT1G47490.1** | **37** | **Non syn** | **E** | **K** |
| 1 | 17733677 | G | A | 26 | 0.93 | 40 | CDS | AT1G48090 | AT1G48090.1 | 3927 | Syn | R | R |
| 1 | 17750954 | G | A | 38 | 1 | 40 | CDS | AT1G48090 | AT1G48090.1 | 748 | Syn | S | S |
| **1** | **17950026** | **G** | **A** | **36** | **0.97** | **40** | **CDS** | **AT1G48540** | **AT1G48540.1** | **963** | **Non syn** | **A** | **T** |
| 1 | 18380468 | G | A | 34 | 1 | 40 | intergenic | | | | | | |
| **1** | **18496569** | **G** | **A** | **37** | **1** | **40** | **CDS** | **AT1G49950** | **AT1G49950.1** | **49** | **Non syn** | **L** | **F** |
| 1 | 18764753 | G | A | 38 | 1 | 40 | intergenic | | | | | | |
| 1 | 18800610 | G | A | 33 | 0.97 | 40 | intronic/noncoding | AT1G50730 | | | | | |
| 1 | 18929372 | G | A | 46 | 0.94 | 40 | CDS | AT1G51070 | AT1G51070.1 | 180 | Syn | P | P |



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| 1 | 19418445 | G | A | 45 | 0.98 | 40 | CDS | AT1G52155 | AT1G52155.1 | 131 | Non syn | C | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 19773895 | G | A | 28 | 0.97 | 40 | CDS | AT1G53050 | AT1G53050.1 | 318 | Syn | L | L |
| 1 | 20010363 | G | A | 35 | 0.97 | 40 | intergenic | | | | | | |
| 1 | 20095463 | G | A | 43 | 0.96 | 40 | intergenic | | | | | | |
| 1 | 20146113 | G | A | 29 | 0.94 | 40 | intergenic | | | | | | |
| 1 | 20199295 | G | A | 29 | 0.91 | 40 | intergenic | | | | | | |
| 1 | 20444833 | G | A | 46 | 0.96 | 40 | intergenic | | | | | | |
| 1 | 20766578 | G | A | 34 | 0.83 | 30 | intergenic | | | | | | |
| 1 | 21033182 | C | T | 53 | 0.93 | 40 | intergenic | | | | | | |
| 1 | 21076283 | C | T | 12 | 0.8 | 30 | CDS | AT1G56290 | AT1G56290.1 | 86 | Non syn | P | S |
| 5 | 26086756 | G | A | 24 | 0.75 | 25 | intronic/non coding | AT5G65280 | | | | | |

**Supplemental Table 1.** SNPs identified by isogenic mapping-by-sequencing. Columns indicate chromosomes (Chr), position in TAIR10 (Position), sequence in referenence (Ref base), alteration in mutant (SNP), number of reads supporting the change (No. reads), fraction of reads supporting the change (concordance), sequence quality score (score), TAIR locus identifier (AGI), TAIR protein identifier ( Protein variant), Position of amino acid affected in protein (Position), Type of mutation (Type), amino acid sequence of TAIR10 reference protein (Ref aa), amino acid in mutant (Change aa). Mutation causing non synonymous amino acid changes are indicated in green letters. Near homozygous mutations potentially changing gene function are indicated in bold.



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| Purpose | Primer | Gene | Sequence (5' to 3') |
|---|---|---|---|
| Genotype | Y040 | *trb1-2* | TTAGCGGAGTCTTGTACCTGC |
| Genotype | Y041 | *trb1-2* | ATGCCACCACAATAAATCTCG |
| Genotype | Y044 | *trb3-2* | ATGGTTCACGAGAAACCTGTG |
| Genotype | Y045 | *trb3-2* | AGGACAACAGATTGATGCACC |
| Genotype | Y093 | *ku70* | TTACTTTGTTGTTTCGGGTGC |
| Genotype | Y094 | *ku70* | CTCTTGGCAAGTACACGCTTC |
| Genotype | Y090 | *tert* | CTAGGACATATCCATCAAGGGCT |
| Genotype | Y091 | *tert* | GAAAGGAAGCTGTATTGCACGAA |
| Cloning | B391 | *TRB1* CDS | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGTGCTCCTAAGCAG |
| Cloning | B392 | *TRB1* CDS | GGGGACCACTTTGTACAAGAAAGCTGGGTCGGCACGGATCATCATTTTGC |
| Cloning | B393 | *TRB1* Promoter | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGCTTTGCTTAAAACGGATTC |
| Cloning | B394 | *TRB1* Promoter | GGGGACCACTTTGTACAAGAAAGCTGGGTCCCCGATTTACGGTCTGCTG |
| Cloning | Y088 | *TRB1* gDNA | GGGGACCACTTTGTACAAGAAAGCTGGGTCATGAAATGACAAAATCATTTG |
| Cloning | B395 | *TRB3* CDS | GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGGAGCTCCAAAGCTG |
| Cloning | B396 | *TRB3* CDS | GGGGACCACTTTGTACAAGAAAGCTGGGTCCCGAGTTTGGCTATGCATTC |
| Cloning | B397 | *TRB3* Promoter | GGGGACAAGTTTGTACAAAAAAGCAGGCTTATTGTTGTTTTGTCAGGGTATCC |
| Cloning | B398 | *TRB3* Promoter | GGGGACCACTTTGTACAAGAAAGCTGGGTCTCACACAATTTTATCACAAAATTC |
| Cloning | Y089 | *TRB3* gDNA | GGGGACCACTTTGTACAAGAAAGCTGGGTCACAAATATAAAGTAGCTAAC |
| Cloning | LHP1F | *LHP1* CDS | GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGAAAGGGGCAAGTGGTGCTG |
| Cloning | LHP1R | *LHP1* CDS | GGGGACCACTTTGTACAAGAAAGCTGGGTCAGGCGTTCGATTGTACTTGAGATG |
| dCAPs | Y396 | *TRB1*RNAR | TGTTTCTCCATTTGTCCTCGA |
| dCAPs | Y387 | *TRB1* RNAF | GTGCTCCTAAGCAGAAATGG |
| RT-PCR | Y129 | *PP2A* | AAATACGCCCAACGAACAAA |
| RT-PCR | Y130 | *PP2A* | CAGCAACGAATTGTGTTTGG |
| RT-PCR | Y528 | *TRB1* MYB F | TGCTCCTAAGCAGAAATGGAC |
| RT-PCR | Y529 | *TRB1* MYB R | GAACGTCCTTTTAACAGCTAAC |
| RT-PCR | Y530 | *TRB3* MYB F | AGCTCCAAAGCTGAAGTGGAC |
| RT-PCR | Y531 | *TRB3* MYBR | TATATTTCTCCATTTGTCCTTG |
| ChIP | Y323 | *AG* | CTTGAAGAGGGCGACAAAAG |
| ChIP | Y324 | *AG* | TTTTAAGGGGCTGGACAAGA |
| ChIP | Y329 | *AP3* | AGGGGGACCAAAGCTAAAAA |
| ChIP | Y330 | *AP3* | TCCACCTGCACTGATTTGAC |
| ChIP | Y302 | *SEP3* | TCTGCCAAGAAAGTTTGATGCT |
| ChIP | Y303 | *SEP3* | CGCCATCTCCACCTTCCATT |



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| | | | |
|---|---|---|---|
| ChIP | Y321 | *FT*-1 | GTGGCGGACAATCCATCTAT |
| ChIP | Y322 | *FT*-1 | AAATATTGGACAGGAGAGCTCAG |
| ChIP | J71 | *FT*-2 | CAAAAGTTTATATTTAGGAGCAGTCAA |
| ChIP | J72 | *FT*-2 | TCAATTCATCATCTCTTCTTTGGA |

**Supplemental Table 2.** List of oligonucleotides used in this study.

## Supplemental References

**Dong, X., Reimer, J., Gobel, U., Engelhorn, J., He, F., Schoof, H., and Turck, F.** (2012). Natural variation of H3K27me3 distribution between two Arabidopsis accessions and its association with flanking transposable elements. Genome biology **13,** R117.

**Engelhorn, J., Reimer, J.J., Leuz, I., Gobel, U., Huettel, B., Farrona, S., and Turck, F.** (2012). DEVELOPMENT-RELATED PcG TARGET IN THE APEX 4 controls leaf margin architecture in Arabidopsis thaliana. Development **139,** 2566-2575.

**Sequeira-Mendes, J., Araguez, I., Peiro, R., Mendez-Giraldez, R., Zhang, X., Jacobsen, S.E., Bastolla, U., and Gutierrez, C.** (2014). The Functional Topography of the Arabidopsis Genome Is Organized in a Reduced Number of Linear Motifs of Chromatin States. The Plant cell **26,** 2351-2366.



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